DEVELOPMENT OF THE POLLEN AND THE EMBRYO SAC IN *CAPSICUM FRUTESCENS* L. VAR. JAPANESE VARIEGATED ORNAMENTAL[1]

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INTRODUCTION

There is great variability in the species of pepper, *Capsicum frutescens* L. The fruits range from the large sweet Bell variety to the small pungent type; the height of the plants varies from eight inches to four feet and there is also wide variation of the flower and fruit in both color and shape. Some varieties are cultivated for their edible fruits and others for their ornamental qualities. Not only is there variability within the species, but variability also occurs within a variety and *C. frutescens* var. Japanese Variegated Ornamental offers an opportunity to observe variation within such a variety.

C. frutescens var. Japanese Variegated Ornamental is about eight to ten inches in height and has variegated—white, purple, and green—leaves. This variegation appears quite early in the growth of the plant, sometimes in the first true leaves, but more often in the second and third leaves. The variegation increases in the later formed leaves until there are some which are predominantly white. This variegation is expressed in an irregular pattern not commonly duplicated. Patterns of variegation occur in flowers and fruits as well as in the leaves. The flowers are typically purple, but occasionally a variegated—white and purple—one is found. The immature fruit color is purple becoming red upon maturing, but sometimes there is an intermediate striping.

This plant is readily propagated by seed, the variegations appearing similar to those of the parent. Most variegated plants are chimeras resulting from a somatic mutation occurring on one of the two outer layers of embryonic tissue of a stem tip; and since most leaves are derived from one or both of these layers, the mutant tissue may appear variously distributed in the mature leaves. Variations of this type can be propagated only vegetatively. Carpels, on the other hand, develop from the corpus or innermost portion of the embryonic stem tip; so, a genetic change which occurs in the first or second layer of the stem tip cannot be transmitted through the carpels (Blaydes, 1953). Since the variegated leaf areas develop in the seedling stages of the pepper variety used in this study, it is apparent that the origin of the variegated state can be explained by assuming the presence of an unstable gene which controls the formation of chlorophyll. If such is the case, each albino area of a given leaf blade represents a group of mutant cells in which the gene was in such a state that it could no longer initiate the formation of green pigment. Each variegated pepper plant then represents an individual in which numerous somatic mutations have occurred, and this occurs as a constant characteristic of this particular variety. For this reason it was felt that this pepper variety could be useful in demonstrating somatic mutations to students dealing with genetics and evolution.

Cochran (1938) reported the development of the pollen and the embryo sac of the Bell or sweet pepper, *Capsicum frutescens* var. *grossus*. He observed that the embryo sac was monosporic. In monosporic development of the embryo sac, the megaspore mother cell divides meiotically and four uninucleate megaspores are formed, each separated by a cell wall. Three megaspores of the tetrad then

[1]From a thesis presented in partial fulfillment of the requirements for the degree Master of Science, The Ohio State University, 1954.

THE OHIO JOURNAL OF SCIENCE 60(1): 8, January, 1960.

disintegrate with the megaspore at the chalazal end surviving. The nucleus of this remaining megaspore divides forming two nuclei which give rise to the two-nucleate embryo sac. These nuclei undergo two divisions, resulting finally in the eight-nucleate embryo sac. Cochran also observed that in *C. frutescens* var. *grossus*, the division of the generative nucleus took place in the pollen tube.

C. frutescens var. Japanese Variegated Ornamental has the same diploid number of chromosomes, twelve pairs, as *C. frutescens* var. *grossus*. Since there is so much variation in the species, *C. frutescens*, and its varieties, I thought that it would be valuable to study the development of the pollen and embryo sac in the Japanese Variegated Ornamental.

MATERIALS AND METHODS

Capsicum frutescens var. Japanese Variegated Ornamental was used for the pollen and the embryo sac studies. Flower buds were killed and fixed in Nawaschin's solution consisting of equal parts of solution A (4 parts of commercial 40% formalin and 1 part water) and solution B (1 gm of chromic acid and 10 cm^3 of acetic acid in 90 cm^3 of water). The material was dehydrated, infiltrated with xylene and paraffin, then embedded in paraffin. Longitudinal sections of the buds were cut 12 μ in thickness, mounted serially and stained with Heidenhain's Iron Hematoxylin. All drawings were made with the aid of a camera lucida.

OBSERVATIONS

In the flower buds of *Capsicum frutescens* var. Japanese Variegated Ornamental, the microspore mother cells differentiate directly from subhypodermal cells of the anther (fig. 1). Following the first meiotic division, no cell walls form between the daughter nuclei (fig. 2, 3). During the second meiotic division, four cells are formed resulting in a tetrad of microspores (fig. 4, 5, 6) that separates with each microspore developing an exine (fig. 7, 8). Following an enlargement of the microspore, the nucleus divides and the two nuclei resulting are unlike (fig. 9, 10). The tube nucleus is spherical and the generative nucleus is crescent-shaped (fig. 10). At the time of dehiscence, each pollen grain contains two linear-shaped sperm nuclei and a larger spherical tube nucleus (fig. 11).

Coinciding with the differentiation of the pollen in the anthers is the development of the embryo sac. A single archesporial cell differentiates in the hypodermal layer of the anatropous ovule (fig. 12) and is distinguished from the other cells by its larger size and its greater affinity for stain. The integument differentiates from the base of the nucellus at the time that the archesporial cell develops without further cell division into the megaspore mother cell (fig. 12). The megaspore mother cell elongates until it is about three times as long as it is wide (fig. 13). The nucleus which is almost as large in diameter as the cell itself contains a deeply staining nucleolus.

After the integument is fully formed, the nucleus of the megaspore mother cell begins to undergo meiotic divisions (fig. 13, 14), the axis of the spindle of the metaphase of the first division being parallel to the long axis of the megaspore mother cell (fig. 15). Following cell wall formation, the resulting cells are called dyads (fig. 16, 17).

When both cells of the dyad divide, the spindles are at oblique angles to the long axes of the cells (fig. 18). Cell walls do not form between the two nuclei of each cell, so meiosis ends with the formation of two binucleated cells (fig. 19). The micropylar binucleated cell becomes disorganized and finally disintegrates leaving the chalazal binucleated cell to become the two-nucleate embryo sac (fig. 20). Maheswari (1950) described this type of megaspore and embryo sac development as bisporic.

The two-nucleate embryo sac increases in length, the nuclei move to opposite ends of the embryo sac and become separated by a large vacuolated region (fig. 21). The cytoplasm surrounding the nuclei is more dense than in any other portion of

the cell. At this point in development, the embryo sac increases in both length and diameter. The chalazal and micropylar nuclei of the two-nucleate embryo sac divide, the resulting four nuclei being smaller than the previous two and orienting at either end of the embryo sac (fig. 22). All four nuclei divide simultaneously, giving rise to eight nuclei, four at each end (fig. 23). One nucleus of each group of four migrates to the center, and at this time the cytoplasm around each of the six remaining nuclei organizes into a cytoplasmic membrane (fig. 24). Sometime before fertilization, the polar nuclei fuse forming the fusion nucleus and a mature embryo sac.

DISCUSSION

The results of the study of the pollen and embryo sac development in *Capsicum frutescens* var. Japanese Variegated Ornamental do not coincide with those of Cochran (1938) for *Capsicum frutescens* var. *grossus*. He found that *C. frutescens* var. *grossus* had a monosporic type development of the embryo sac, while I have found a bisporic type development in *C. frutescens* var. Japanese Ornamental. Such variations as to embryo sac type within a species have been reported for *Erythronium americanum* by Hague in 1950 and *Ulmus fulva*, *U. racemosa* and *U. glabra* by Walker in 1950 (Maheshwari, 1950).

Cochran also observed for *C. frutescens* var. *grossus* that the division of the generative nucleus took place in the pollen tube, and my results show that particular division having taken place in the pollen grain of *C. frutescens* var. Japanese Variegated Ornamental.

SUMMARY

1. *Capsicum frutescens* var. Japanese Variegated Ornamental is a plant with variegated—white, purple and green—leaves and occasional striped flowers and fruits.

2. Every individual of *C. frutescens* var. Japanese Variegated Ornamental is regularly propogated by seed. This is most unusual for variegated plants, because variegation is not usually transmitted by gametes.

EXPLANATION OF FIGURES IN PLATE I*

1. The microspore mother cell (Approx. ×350).
2. The first meiotic division of the microspore mother cell (Approx. ×890).
3. The daughter nuclei following the first meiotic division of the microspore mother cell (Approx. ×410).
4. The second meiotic division of the microspore mother cell (Approx. ×710).
5. The tetrad of microspores (Approx. ×830).
6. The tetrad of microspores separating (Approx. ×790).
7. The microspores after the exine developed (Approx. ×770).
8. The exine of the microspores (Approx. ×470).
9. The microspore before the nucelar division (Approx. ×770).
10. The binucleate pollen grain (Approx. ×770).
11. The trinucleate pollen grain (Approx. ×750).
12. The megaspore mother cell in the anatropus ovule (Approx. ×170).
13. Prophase of the first meiotic division of the megaspore mother cell (Approx. ×820).
14. Prometaphase of the first meiotic division of the megaspore mother cell (Approx. ×710).
15. Metaphase of the first meiotic division of the megaspore mother cell (Approx. ×810).
16. The dyad before cell walls form (Approx. ×810).
17. The dyad resulting from the first meiotic division of the megaspore mother cell (Approx. ×770).
18. The second meiotic division of the megaspore mother cell (Approx. ×770).
19. The two binucleated cells (Approx. ×860).
20. The micropylar binucleated cell disintegrating (Approx. ×810).
21. The two-nucleate embryo sac (Approx. ×410).

*All figures in Plate I are photomicrographs.

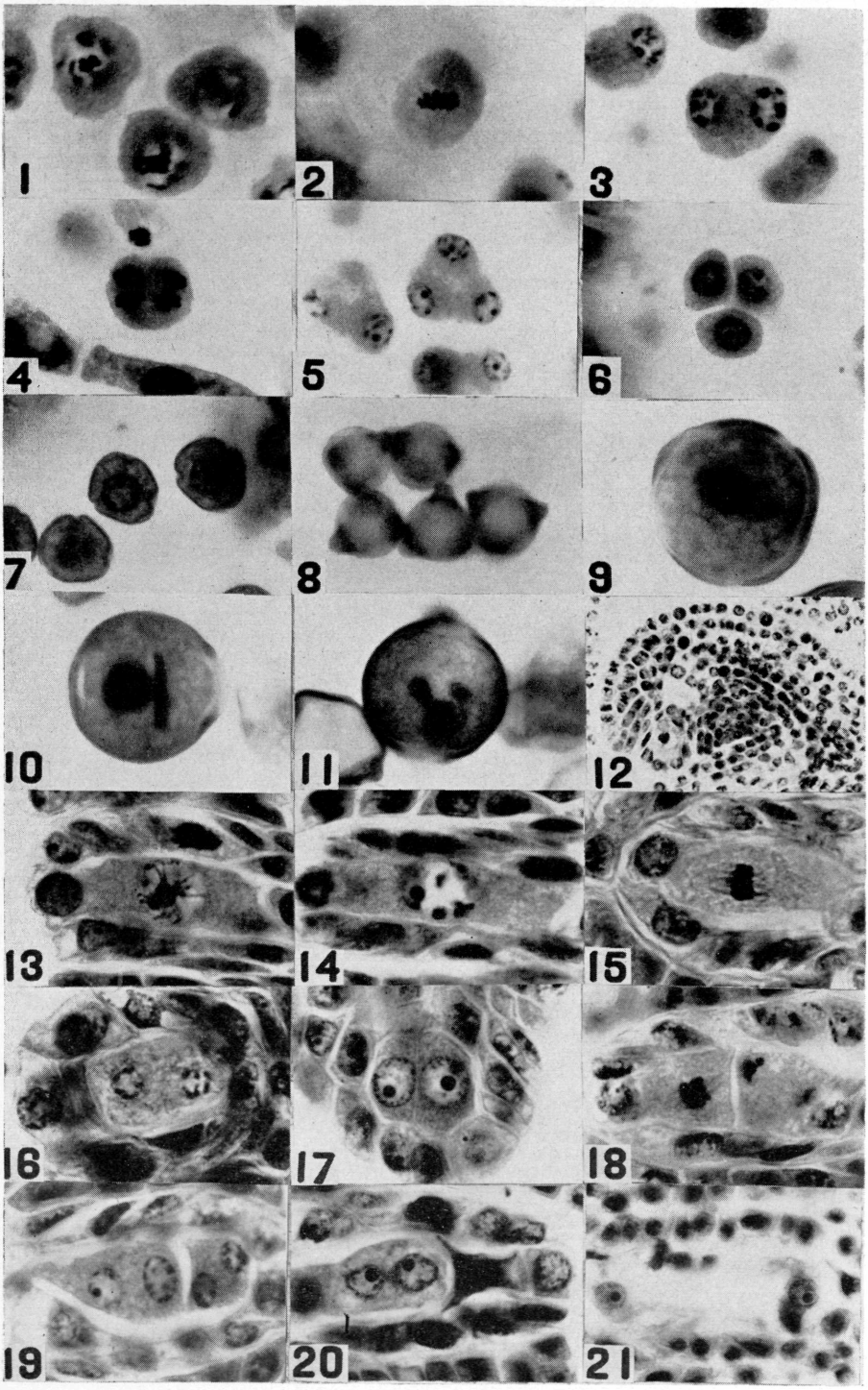

PLATE I

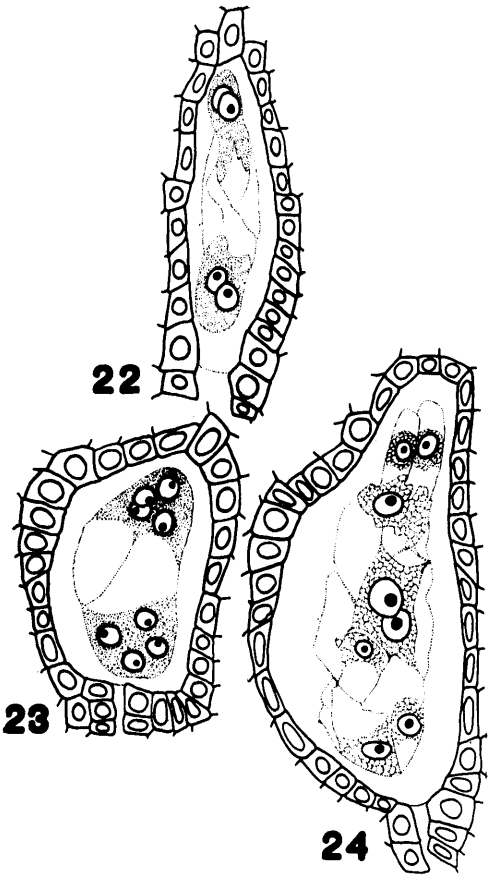

FIGURE 22. Four-nucleate embryo sac (Approx. × 300).

FIGURE 23. Eight-nucleate embryo sac (Approx. × 300).

FIGURE 24. Eight-nucleate embryo sac after the two polar nuclei migrate (Approx. × 300).

3. Each variegated area of a leaf may be explained by assuming that a somatic mutation occurred in a cell which becomes the forerunner of each albino area or patch of cells.

4. The chalazal binucleated cell becomes the two-nucleate embryo sac, hence the development is bisporic. This was not expected since the monosporic development had previously been described for the Bell pepper, another variety of the same species.

5. The generative nucleus divides before the pollen germinates, resulting in trinucleate pollen. This was unlike the development in the Bell pepper where the pollen is shed in the binucleate condition.

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